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Abstract: Drive genes are genetic elements that manipulate the 50% ratio of Mendelian inheritance in their own favour, allowing them to rapidly propagate through populations. The action of drive genes is often hidden, making detection and identification inherently difficult. Yet drive genes can have profound evolutionary consequences for the populations that harbour them: most known drivers are detrimental to organismal gamete development, reproduction and survival. In this study, we identified the presence of a well-known drive gene called t haplotype post hoc in eight replicate selection lines of house mice that had been evolving under enforced monandry or polyandry for 20 generations. Previous work on these selection lines reported an increase in sperm competitive ability in males evolving under polyandry. Here, we show that this evolutionary response can be partly attributed to gene drive. We demonstrate that drive-carrying males are substantially compromised in their sperm competitive ability. As a consequence, we found that t frequencies declined significantly in the polyandrous lines while remaining at stable, high levels in the monandrous lines. For the first time in a vertebrate, we thus provide direct experimental evidence that the mating system of a species can have important repercussions on the spread of drive genes over evolutionary relevant timescales. Moreover, our work highlights how the covert action of drive genes can have major, potentially unintended impact on our study systems.

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Sperm competition suppresses gene drive among experimentally evolving populations of house mice

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Abstract

Drive genes are genetic elements that manipulate the 50% ratio of Mendelian inheritance in their own favour, allowing them to rapidly propagate through populations. The action of drive genes is often hidden, making detection and identification inherently difficult. Yet drive genes can have profound evolutionary consequences for the populations that harbour them: most known drivers are detrimental to organismal gamete development, reproduction and survival. In this study, we identified the presence of a well-known drive gene called *t* haplotype *post-hoc* in eight replicate selection lines of house mice that had been evolving under enforced monandry or polyandry for 20 generations. Previous work on these selection lines reported an increase in sperm competitive ability in males evolving under polyandry. Here, we show that this evolutionary response can be partly attributed to gene drive. We demonstrate that drive-carrying males are substantially compromised in their sperm competitive ability. As a consequence, we found that *t* frequencies declined significantly in the polyandrous lines while remaining at stable, high levels in the monandrous lines. For the first time in a vertebrate, we thus provide direct experimental evidence that the mating system of a species can have important repercussions on the spread of drive genes over evolutionary relevant time scales. Moreover, our work highlights how the covert action of drive genes can have major, potentially unintended impact on our study systems.

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Introduction

Natural selection is typically thought of as a process that will favour genes that improve the fitness of the organisms that harbour them. Driving elements convincingly remind us that this rule can be systematically broken. These stretches of DNA are often detrimental to organismal survival and reproduction (Burt & Trivers 2006). By manipulating gametogenesis, drive elements bias the fair 50:50 ratio of Mendelian inheritance in their favour (Burt & Trivers 2006). The resulting systematic transmission advantage at the level of the gamete, termed gene drive, allows drive elements to rapidly propagate in populations *in spite* of the potential costs they impose on the organism as a whole. Previous research suggests that gene drive is not only widespread across diploid life (Burt & Trivers 2006), but that it can have profound implications for populations that harbour driving elements, affecting a wide range of phenomena from gametogenesis, fertility, and mating behaviour to population survival and speciation (Lindholm *et al.* 2016).

The action of drive genes is often hidden, making detection and identification inherently difficult. As a result, our knowledge of drive biology is still fairly limited and centred around few genetic model species (*Drosophila,* mice, and *C. elegans*). Not surprisingly, one of the best-understood drive systems, called *t* haplotype, is found in house mice (*Mus musculus*). The *t* haplotype comprises a whole cluster of genes that occupies about one third of chromosome 17. It is detrimental to its carriers: *t/t* homozygotes die from recessive lethal mutations during embryogenesis (Ardlie & Silver 1996; Lindholm *et al.* 2013). The fatal condition of *t/t* homozygotes seemingly predicts rapid extinction of the *t* haplotype. However, by distorting the transmission ratio, heterozygous males transmit the *t* haplotype to up to 90% of their offspring, which is significantly higher than the expected 50% transmission under Mendelian inheritance (Ardlie & Silver 1996). The genetic mechanism behind this drive effect is relatively well understood: +/*t* heterozygote males produce + and *t* gametes in equal proportions, but a set of distorter loci interferes with the flagellar function of all sperm (+ and *t*) during spermatogenesis, while *t* sperm swimming ability is locally restored by a responder (akin to a poison-antidote system (Herrmann &

Bauer 2012)). The consistent advantage at the level of the gamete has allowed *t* haplotypes to persist in house mouse populations around the world for more than 1.5 million years (Hammer & Silver 1993).

A key challenge for drive research has been to understand the factors that determine the frequency of drive genes in natural populations (Lindholm *et al.* 2016). No system epitomises our lack in understanding drive dynamics better than the *t* haplotype, where the quest to explain naturally occurring frequencies dates back more than half a century (reviewed by Ardlie (1998). At its heart lies a puzzle that has been termed the '*t* frequency paradox', describing a discrepancy between theory and observation. An early theoretical study predicted that, based on drive and homozygote lethality, approximately 70% of the individuals in a population should be *t* carriers (Bruck 1957). However, empirical measures from natural mouse populations around the world have shown that *t* frequencies are in fact considerably lower than predicted, typically ranging between 5% and 30% (Lenington *et al.* 1988; Ardlie & Silver 1998; Huang *et al.* 2001; Dod *et al.* 2003; Manser *et al.* 2011). The discrepancy between theoretical prediction and observation is suggestive of the presence of evolutionary mechanisms that suppress drive. Over the years, a multitude of propositions to resolve the *t* paradox have been brought forward, ranging from the evolution of genes that directly interfere with the mechanism at the molecular level (Charlesworth & Hartl 1978; Gummere et al. 1986) to interdemic selection at the population level (Lewontin & Dunn 1960; Nunney 1993). However, most of the propositions remain speculative because they rely on parameters for which solid estimates are largely missing (Ardlie 1998; Burt & Trivers 2006).

A mechanism of drive suppression that has received considerable attention over the past few years is sperm competition, which occurs when females mate with two or more males in a single reproductive event (referred to as polyandry, see Wedell (2013) for a comprehensive review on the relationship between sperm competition and gene drive). The hypothesis of sperm competition as a potential suppressor of drive, originally put forward by Haig & Bergstrom (1995), is based on a simple premise. Most known distorter systems drive through males, and often involve the killing of those sperm that do

not carry the drive chromosome. Such targeted killing makes drive-carrying sperm, by definition, successful against rival sperm *within* a male's ejaculate, yet typically result in drive-males producing fewer viable sperm (Price & Wedell 2008). As a result, the fewer sperm of drive-carrying males are often outcompeted by the more numerous sperm of non-driving males in sperm competition *between* ejaculates. Controlled sperm competition experiments in a number of taxa have confirmed this conjecture: driving elements are often detrimental to a male's sperm competitiveness — including impaired success of those sperm that carry the drive element (Wilkinson & Fry 2001; Atlan *et al.* 2004; Price & Wedell 2008; Sutter & Lindholm 2015). The link between drive and male sperm competitive ability bears two interesting implications. First, we predict that sperm competition will suppress drive frequency in populations where female multiple mating is common. In the context of the *t* haplotype, this may help us resolve the *t* frequency paradox (Manser *et al.* 2011). Second, if a female can 'invite' sperm competition to reduce the proportion of offspring inheriting a harmful drive gene, we can expect coevolution between drive and the tendency of females to mate multiply (Holman *et al.* 2015). Indeed, drive has been shown to promote polyandry rates in a selection experiment in *Drosophila pseudoobscura* (Price *et al.* 2008). Gene drive has therefore been proposed as one of many explanations for the evolution of polyandry (Wedell 2013).

In the present study, we directly tested whether sperm competition can suppress gene drive by conducting a *post-hoc* analysis of individuals from experimental populations of wild-derived house mice (*Mus musculus domesticus*) that had been maintained under either a strict monandrous or polyandrous mating regime for 20 generations (Firman & Simmons 2011). Earlier work had revealed that males from these selection lines had diverged in the predicted direction in sperm number and quality (Firman *et al.* 2011), and in sperm competitiveness (Firman & Simmons 2011). Here, we first established that the *t* haplotype was present in all eight replicate lines. Following this, we asked i) how the *t* haplotype affected sperm competitiveness both *within* and *between* ejaculates, ii) how much of the selective divergence in sperm competitiveness between monandrous and polyandrous lines was *t* haplotype related; iii) how the *t* haplotype affected female fitness, and iv) how the mating regime (monandry

112 versus polyandry) affected *t* frequency dynamics among the selection lines. In so doing we have
113 provided unequivocal evidence that sperm competition can act as an effective suppressor of drive
114 systems over generations.

115 Material and Methods

116 The Study System

117 ***Selection Experiment.*** For a detailed description of the mating regimes associated with the selection
118 experiment see Firman & Simmons (2011). In brief, a total of eight replicate lines of mice were subjected
119 to either a monandrous or a polyandrous mating regime over 20 successive generations. Four replicate
120 lines were mated monandrously (*M*-lines) via a middle-class neighbourhood design. Each *M*-line
121 consisted of 18 males and 18 females and each fecund pair contributed one randomly selected male and
122 female to the next generation. Hence, all individuals of *M*-lines had the same fitness, eliminating both
123 natural and sexual selection. Four replicate lines, again consisting of 18 females and 18 males, were
124 mated polyandrously (*P*-lines). Each *P*-line female was mated to three different males (see Fig. 1 in
125 Firman & Simmons (2010) for an illustration of the mating design), which is relevant to mating
126 frequencies observed in nature (Firman & Simmons 2008a). Hence, as opposed to the *M*-lines, *P*-line
127 males competed for fertilizations, and the number of males who contributed to successive generations
128 was determined by the relative fertilization success of a given male. In other words, postmating sexual
129 selection operated in the *P*-lines, while it did not in the *M*-lines. As in the *M*-lines, one male and one
130 female offspring per family were randomly selected to contribute to the subsequent generation,
131 resulting in the removal of postnatal natural and premating sexual selection. Mice that entered the
132 selection experiment were kept under monandrous mating conditions for approximately 30 generations
133 prior to this experiment (Firman & Simmons (2010).

The t haplotype in the selection lines. In this study, we genotyped tissue samples of individuals from the selection lines for the presence of the *t* haplotype. In total, 1092 individuals from generations 0, 12, 16, and 19 were typed at the *Hba-ps4* locus (Hammer *et al.* 1989). We found that the *t* haplotype occurred at considerable frequencies in all eight selection lines. Of the 1092 samples none were genotyped as *t/t* homozygous, indicating that *t* haplotype present in the selection lines carried a recessive lethal mutation.

Measuring *t* Sperm Competitiveness

Sperm competition experiment. To estimate how the *t* haplotype affected a male's sperm competitiveness, we used data from a sperm competition experiment that had been conducted previously on mice from the 12th generation of the selection lines. Firman & Simmons (2011) provide a detailed description of the experimental method. Briefly, two males — one *M*-line and one *P*-line male — were mated to *M*-line and *P*-line females using a semi-factorial design, creating a total of 32 ordered male combinations (16 *M x P* and 16 *P x M*). Specific male combinations were randomly assigned to females of either selection history. To avoid confounding effects of coevolution within lines, matings between males and females from the same replicate lines were avoided. The entire design was replicated, resulting in 64 experimental matings. Females deemed to be in oestrus were placed in a male's box and checked half-hourly for the presence of a mating plug. The plug was removed upon detection and females were then paired with a second male. Females were again checked in half-hour intervals for the presence of a mating plug, which was removed after a successful second mating had been achieved. Fourteen days after mating, females were sacrificed and dissected, and embryos were removed from the reproductive tract for genetic paternity analysis (see Firman & Simmons (2011) for methods on paternity analysis). In the present study, we determined the *t* genotype of the parents (mothers and potential sires: 192) and offspring (495). We were successful in genotyping 190 parents and 493 embryos. As none of the 493 embryos were *t/t* homozygous, we conjecture that the genotypic outcomes of each mating cross were measured at an embryonic stage at which *t/t* lethal embryos were

already resorbed. Paternity outcome and *t* genotype of parents and offspring of the competitive mating crosses allowed us to estimate two key measures of *t* sperm competitiveness, drive *d* and sperm competitiveness cost *c.*

Estimating Gene Drive. Parameter *d* defines drive, i.e. the probability that a genetic offspring of a $+/t$ male inherits the *t* allele. Since *+/t* males produce + and *t* in equal proportions, with *+* sperm motility being compromised at a later stage of spermatogenesis, parameter *d* can also be thought of as a measure of *t* sperm competitiveness *within* a *+/t* male. If $d = 0.5$, sperm transmission ratio is perfectly Mendelian, i.e. both + and *t* haplotypes have identical probability of transmission through $+/t$ hetorozygotes (Null hypothesis). If *d=1, +/t* males transmit the *t* haplotype to all offspring.

Ideally, drive is estimated in the absence of sperm competition between males, that is in monandrous crosses that involve *+/t* males only. However, in our sperm competition experiment the mating crosses consistently involved two males that competed for fertilizations. Under the assumption that the genotypic outcome *within* the subset of offspring sired by a given male is independent of sperm competitive effects *between* ejaculates, we treated each subset sired by a given male as if mated monandrously. Accordingly, we used P_{within}, defined as the proportion of *+/t* heterozygotes (based on *t* genotype information) among all viable offspring sired by a given *+/t* male (based on paternity information), as our response variable to estimate drive parameter *d*.

Note that, for a given value of drive *d*, P_{within} will depend on the female genotype. In a cross between a *+/+* female and a *+/t* male, male drive alone determines the proportion of *+/t* offspring, and we have $P_{within} = d$. In a cross between *+/t* female and a *+/t* male, both males and females may provide *t* gametes and a fraction $\frac{d}{2}$ will die during embryogenesis due to *t/t* lethal effects, resulting in $P_{within} = \frac{1/2}{1-d/2}$. In order to simultaneously derive drive estimates across both mating contexts, we wrote a customized likelihood function that makes use of the two expressions above (see Table S1). This approach allowed us to find the parameter value *d* that best fitted the observed P_{within} values using a

maximum likelihood estimation (using the `mle2` function in the `bbmle` package in *R* (Bolker & Team 2014; R Core Team 2015)). The proportion of *+/t* offspring was assumed to follow a beta-binomial distribution, which allowed us to account for potential overdispersion with parameter θ. In a full model, we allowed drive estimates to vary as a function of female genotype (*+/t* or *+/+*), the mating order of the male (1^{st} or 2^{nd}), male selection history (*M* or *P*) and their interaction. Competing models of decreasing complexity were then compared based on *AIC* values.

Estimating Sperm Competitiveness. Parameter *r* measures the relative competitiveness of *+/t* males compared to +/+ males (whose competitiveness equals unity). *r* can thus be interpreted as the competitiveness of a *+/t* male's sperm in competition *between* males, and corresponds to the measure of sperm precedence or loading of the sperm raffle standard to sperm competition theory (Parker 1990). If *r=1*, *+/+* and *+/t* males do not differ in their sperm competitive ability (Null hypothesis 1). However, *+/t* may have reduced sperm competitiveness as a *direct* consequence of drive, because the *t* haplotype renders a considerable fraction of a *+/t* male's sperm dysfunctional. If a male's competitiveness were proportional to his number of viable sperm, competitiveness r can be expressed directly as a function of drive parameter *d*, with $r[d] = \frac{0.5}{d}$ (Null hypothesis 2). For example, if drive is complete ($d = 1$), half of a *+/t* male's sperm (i.e. *all* + sperm) will be killed, and as a result his sperm competitiveness is expected to be halved (since $r[1] = 0.5$).

To estimate between ejaculate parameter *r*, we analysed the proportion of viable offspring sired by a *+/t* male when competing against a *+/+* male, denoted by $P_{between}$, as a response variable. Due to embryo mortality and different *t* frequency among female gametes, we again expect different values $P_{between}$ depending on female genotype for a given levels of parameters *r* and *d* (see Table S1). To find the best estimate of parameter $\hat{r}$ given the observed data across mating context, we again wrote a customised likelihood function which we optimised using maximum likelihood with the `mle2` function (Bolker & Team 2014). $P_{between}$ was again assumed to follow a beta-binomial distribution. In a full model, we allowed *r* to vary as a function of *+/t* male order (1^{st} or 2^{nd}), male selection history (*M* and *P*), their

interaction, and female genotype (*+/+ or +/t*). Again, we performed model selection in a backward fashion using *AIC* values.

Quantifying the Importance of Male Selection History

The previous analysis is specifically tailored towards estimating sperm competitiveness of *+/t* males, and thus focused on competitive crosses that involved both a +/+ and a +/t male. To more precisely quantify the importance of the *t* haplotype relative to selection on male sperm competitiveness elsewhere in the genome (as reported in Firman & Simmons (2011)), we conducted an additional analysis where sperm competition outcomes were examined across *all eight* combinations of male genotype and selection history (including crosses between males of the same *t* genotype, see Fig. S1). We analysed the paternity of the second male P_2 as a function of the selection history (*M* or *P*) and *t* genotype of the second male (*+/+ or +/t*), the *t* genotype of the first male (*+/+ or +/t*), as well as all two- and three-way interactions in a generalised linear model with a quasi-binomial error and a logit link. We performed systematic model selection based on *QAIC* (correcting for overdispersion) values using the `dredge` function in R (within the `MuMIn` package (Bartoń 2015)). To quantify the relative importance of male selection history versus the *t* genotype on male sperm competitiveness, we considered the deviance explained by either factor when added sequentially to a given model.

Female Fitness Costs

Due to *t/t* embryo mortality, we expected *+/t* females to suffer from a reduced litter size in any mating cross with successful *+/t* fertilization. We analysed litter sizes as a function of the proportion of offspring sired by a *+/t* male and of female genotype in a generalized linear model (GLM) using an identity link function and a Poisson error distribution. We further performed a power analysis in order to assess whether the sample size was sufficiently large to detect a litter size effect (see Supplementary Text S2). Importantly, litter size losses, even if present, will not affect *t* frequency dynamics in the

selection lines since every female contributed a standardized number of two offspring to the subsequent generation.

t Frequency Dynamics in the Selection Lines

To test whether the mating regime (monandry vs. polyandry) affected t frequency dynamics in the selection lines, we analysed the observed frequency of $+/t$ genotypes $\hat{y}$ as a function of treatment (M or P), time (generations $g \in \{0,12,16,19\}$), and their interaction in a generalized linear mixed effects model (GLMM) using a logit link function and a binomial error distribution. We fitted random intercept and slope for each selection line to allow for variation among the eight replicate lines, both in terms of starting frequency (random intercept) and frequency change (random slope).

Results

Measuring *t* Sperm Competitiveness

Levels of drive, d In 53 cases, a $+/t$ male successfully sired at least one genotyped embryo within a litter, allowing us to calculate the proportion of $+/t$ offspring among his progeny P_{within} (see Fig. 1a). According to the model selection procedure, drive levels d did not systematically vary as a function of mating order, male selection history, or female genotype (see Table S2). In the minimal adequate model, we were thus left with a context-independent drive estimate of $\hat{d} = 0.78$ (95\% CI: *[0.66,0.86]*). In terms of within male sperm competitiveness, this means that a t sperm 3.45 times ($\frac{d}{1-d}$) more likely to fertilise an egg that + sperm. As suggested by the confidence interval, this estimate significantly deviated from Mendelian expectations ($z=4.43$, $P<0.001$ against $H_0: d = 0.5$).

Sperm competitiveness, r In 34 experimental mating crosses, a $+/t$ male competed against a $+/+$ male, allowing us to calculate the $+/t$ male's fertilization success $P_{between}$ (see Fig.1b). Based on model selection, neither male selection history nor female genotype had an influence on sperm

competitiveness *r* (see Table S3). Mating order, on the other hand, played an important role with the first male achieving a significantly larger share of paternity in a given litter (*z=2.98, P<0.01*). More importantly, *+/t* sperm competitiveness was substantially reduced compared to *+/+* males: corrected for the mating order effect, the maximum likelihood method yielded a parameter estimate of $\hat{r} = 0.24$ (95\% CI: *[0.12,0.45]*). In other words, a +/+ male is more than 4 times ($\hat{r}^{-1}$) more likely to fertilise a given egg than a +/t male. This estimate was significantly different from both a naïve Null hypothesis (*z=4.24, P<0.001* against $H_0: r = 1$) and the Null hypothesis that corrects for *t* related killing of + sperm ($H_0: r = \frac{0.5}{\hat{d}} = 0.64$, two sample t test comparing observed and expected (Null) distribution: *t=2.87, P<0.01*).

Quantifying the Importance of Male Selection History

The analysis confirmed male *t* genotype as a strong predictor of male sperm competitiveness (see Fig. S1). All five best models included both *t* genotype of the first and the second male (as well as their interaction in 4/5 cases, see Table S4), with *t* haplotypes drastically reducing a given male's fertilization probability. In contrast to the analysis above, all five best models suggest that males which had evolved under the polyandrous regime (*P* lines) had an increased fertilisation success when compared to males that originated from monandrous *M* lines (weighed average and 95% CI across all models: 1.44 (0.61, 2.27), $z = 3.4$, $P < 0.001$, see Fig. S1). According to the analysis of deviance table of model 16, male selection history explained 14.8% of the deviance in fertilisation success, while *t* genotype of the first and second male (and their interaction) cumulatively explained 27.4% of deviance. Note that model 16 is only the second best model based on *QAIC* (see Tab. S4), but allowed us to *separately* quantify the effect of the two factors as it does not contain any interaction between them.

Female Fitness Costs

Fig. 1c illustrates litter sizes as a function of the proportion of the litter that was sired by a *+/t* male. Surprisingly, the proportion of embryos sired by +/*t* males did not affect litter sizes of +/*t* females: both

explanatory variables (female genotype, proportion *+/t* male sired) were removed during model selection (see Table S5), leaving us with a simple intercept model with an average litter size of 7.77 (95% CI: *[7.10,8.49]*). A power analysis suggested that the sample size available here would be sufficient to detect the expected litter size reductions due to *t/t* mortality if present (see Text S2 and Fig. S2).

t Frequency Dynamics in the Selection Lines

Figure 2 shows the observed and predicted *t* frequency dynamics as a function of the mating regime (monandry vs. polyandry). Note that the *t* haplotype frequency is simply half the frequency of +/t genotypes as *t* haplotypes can only occur in *+/t* heterozygotes due to *t/t* lethality. The GLMM indicated that the frequencies at generation 0 did not differ between the two selective treatments (z=–0.35, P=0.72). We thus fitted a simpler model (ΔAIC=-1.8) that only estimates one intercept for both selection treatments (the prediction for *t* frequency at generation 0 was 0.36 (95% CI: [0.31, 0.40], range of values across the 8 lines: [0.23, 0.50]). The model revealed that t haplotype frequency remained constant in the monandrous lines (slope and 95% CI per generation on the logit scale: 0.010 [–0.03, 0.05], z=0.48, P=0.63), while decreasing significantly in the polyandrous lines (slope and 95% CI per generation on the logit scale: –0.066 [–0.10, –0.03], z=–3.47, P<0.001), resulting in a significant interaction between selective treatment and time (estimate and 95% CI of the difference in slopes between the monandrous and polyandrous lines: –0.076 [–0.110, –0.042], z=–4.54, P<0.001).

Discussion

Our study validates two key predictions regarding polyandry and sperm competition as a suppressor of gene drive in the *t* haplotype system of house mice. Firstly, we show that the *t* haplotype heavily compromised +/*t* males in their sperm competitive ability when competing against +/+ males. Secondly, we show that this systematic +/*t* male disadvantage in sperm competition significantly affected *t* haplotype dynamics over 20 generations; *t* allele frequencies declined significantly among selection

lines that were maintained under a strict polyandrous mating regime, and yet remained at high levels among selection lines in which mice were bred monandrously.

Gene drive compromises male sperm competitiveness We have shown that +/*t* males are severely compromised in their sperm competitive ability. Our best estimate of *r=0.24* suggests that +/*t* males, on average, managed to sire only 19% of a litter when competing against a +/+ male (prior to embryo mortality). The effect of the *t* haplotype on sperm competitiveness has rarely been measured directly, and most previous estimates are based on extremely limited sample sizes. A very good estimate of *+/t* male sperm competitiveness based on a substantial sample size was provided recently by Sutter and Lindholm (2015), who reported a *+/t* male paternity share as low as 11% in a *t* haplotype variant from Switzerland. Older studies based on different methods and smaller samples report similar levels of *+/t* male sperm disadvantage, with paternity share ranging between 17% and 22% (Olds-Clarke & Peitz 1986; Ardlie & Silver 1996; Manser *et al.* 2011). Strikingly, all estimates suggest that +/*t* males are worse sperm competitors than one would expect based on the killing of wildtype sperm *alone* (Null hypothesis 2). We see two potential explanations for this elevated discrepancy in sperm competitiveness. First, it could be a non-adaptive by-product of the genetic mechanism causing drive. If the *t* haplotype's poison antidote system is not perfectly fine-tuned, it may not only affect + sperm in their swimming ability, but also compromise the fertilizing competency of *t* sperm (Sutter & Lindholm 2015). Second, the drastically reduced sperm quality of drive males could be the (adaptive) product of differential strategic investment in sperm traits by *+/+* and *+/t* males. Engqvist (2012) has analysed differential ejaculate investment in a situation where males systematically differ in sperm competitiveness using a game-theoretical framework. The model finds that under certain circumstances (large discrepancy in male sperm quality, low risk of sperm competition, low frequency of low quality males), the poor sperm competitors (in this case the drive males) fight a losing battle if investing in sperm traits, as they will always be outcompeted by the high quality males. In this case, low quality (drive) males will be better off by allocating resources into traits that maximise mating success. The relative importance of these two, mutually non-exclusive hypotheses needs to be investigated further. As a first step, Sutter &

Lindholm (2015) have measured the size of male reproductive organs (testes and epididymides) and found no difference between +/+ and +/t males.

The systematic disadvantage of drive carrying males in sperm competition against other males is not an isolated observation for *t* haplotypes, but a recurring pattern across drive systems (Price & Wedell 2008). For example, similar reductions in the paternity share of drive males have been reported among sex chromosome drive systems of invertebrates (Wilkinson & Fry 2001; Atlan *et al.* 2004; Price *et al.* 2008) and plants (Taylor *et al.* 1999). This is likely a consequence of similarities in the drive mechanisms, which often use sperm as the target of their attack.

Absence of female fitness costs We found that +/*t* females did not suffer any litter losses when fertilized by +/*t* males. This is surprising, if one considers that *t/t* embryos die during embryogenesis. A power analysis showed that our sample sizes were sufficient to detect expected litter losses given observed drive levels $\hat{d}$. We can only speculate why this was not the case here. One possibility is that the mice used in the selection experiment carried a *t* haplotype variant with a lethal mutation that acts early during embryonic development, i.e. before the embryos are implanted into the uterus (which occurs by day 5 of pregnancy (Kaufman 1992)). At least 16 different *t* haplotypes have so far been identified (Klein *et al.* 1984), each containing lethal mutations that act at different stages of embryonic development. One complementation group (t_{12}) has been shown to act before day 4 of pregnancy (Bennett 1975). Interestingly, early studies suggest that this *t* haplotype variant is associated with drive levels around 75% (Smith 1956; McGrath & Hillman 1980), a value that is strikingly close to our estimates here. However, the same studies also report reduced litter sizes in crosses between +/t_{12} heterozygotes. If more embryos are produced than can be implanted, females might use cryptic mechanisms to select embryos that are developing normally (Kozlowski & Stearns 1989), thereby avoiding *t/t* related litter losses. A previous analysis revealed that females from the selection lines did in fact, on average, produce more ova in a given cycle than live births (approx. ova number: *P*-lines = 14, *M*-lines = 16 (Firman & Simmons 2011)). It is important to note that the absence of a reduction in litter size, irrespective of its

causes, will not affect our *t* frequency expectations in the selection lines, because the litter size of all females was standardised to two.

Does sperm competition explain low t frequency in natural populations? One important consequence of the systematic disadvantage of drive-carrying males is the effect of polyandry on drive frequency dynamics. Understanding the factors that explain drive frequency dynamics in natural populations is a longstanding focus in drive research, in the *t* haplotype system specifically (Ardlie 1998), but also in drive systems generally (Lindholm *et al.* 2016). This is the first study to directly examine the impact of female mating frequency on the dynamics of the *t*-haplotype under controlled experimental conditions over multiple generations. We have shown that the imposition of polyandry in four independent selection lines resulted in a significant decrease in the frequency of the *t* haplotype, and thereby provide the first direct empirical evidence that polyandry functions as a drive frequency suppressor in this system.

In natural populations, *t* haplotypes are typically found at markedly lower frequency than predicted by theory (referred to as the *t* frequency paradox). Empirical evidence from both laboratory and natural populations suggest that house mice are often polyandrous, with multiple paternity rates ranging between 20 and 30% (Dean *et al.* 2006; Firman & Simmons 2008b; Manser *et al.* 2011; Thonhauser *et al.* 2014; Manser *et al.* 2015). The results of this investigation, taken together with the rates of polyandry reported among natural populations, suggests that female remating behaviour may be an important factor in explaining the *t* frequency paradox. Moreover, polyandry mediated drive suppression may explain an additional pattern found in natural populations. *t* frequencies are typically negatively correlated with population size (Ardlie 1998), precisely the pattern one would expect if polyandry rates increase with increasing population size, i.e. as the number of mating opportunities increases (Dean *et al.* 2006).

Finally, our study is a striking example of how the hidden action of a drive system can, unknowingly, have large-scale effects in an experimental system. Previous work published on these selection lines reported an increased sperm competitive ability and sperm quality of males from the polyandrous selection lines (Firman & Simmons 2010; 2011). Here, we show that 27% of the variation in male sperm competitiveness can be attributed to the *t* haplotype, thus providing a mechanistic explanation for the previously published effects. However, our analyses also suggest that, once corrected for *t* related effect, male selective background still accounted for about 15% of the total variance in sperm competitiveness. This suggests selection on standing genetic variation in traits affecting sperm competitiveness that are independent of the *t* allele. Due to the absence of obvious phenotypic effects, drive systems are often inherently difficult to detect, requiring in depth cytological or genomic work over multiple generations (Burt & Trivers 2006). Our experiments were conducted on one of the best-studied, genetic model organisms, making the identification of a gene drive relatively easy. As a result of the genomic revolution, new drive systems across a broad range of taxa are being described at an ever-faster rate. These discoveries suggest that drive is not a rare, isolated phenomenon but widespread across diploid life (Burt & Trivers 2006). Our study demonstrates that uncovering the hidden action of these genetic outlaws is indeed a worthwhile endeavour. At worst, gene drivers may help us identify hidden, unintended side effects in our study systems. At best, gene drivers may provide us with a deeper, more mechanistic understanding of the evolutionary processes under examination, as was the case here.

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493 ***Data accessibility.*** Parentage, *t* genotype, and litter sizes of competitive mating crosses and frequency
494 of *t* genotypes in selection lines will be archived in the Dryad digital repository.

495 ***Author contributions.*** This study resulted from a collaboration between the Manser/Lindholm group, who
496 study gene drive in house mice in the laboratory and the field, and the Firman/Simmons group, who ran a
497 long term experimental evolution study of polyandry in house mice without knowledge that a gene driver
498 was present in their lines.

Figures Legends

Figure 1. (a) Proportion of *+/t* heterozygotes among all viable offspring sired by a given *+/t* male P_{within} as a function of female genotype. Square dots and lines show model predictions and 95% CI based on the maximum likelihood estimation of parameter *d*. (b) Paternity share of a $+/t$ male when competing against +/+ male $P_{between}$ as a function of female genotype. Square dots and lines show model predictions including 95% CI based on the maximum likelihood estimation of parameter *c*. (c) Litter sizes as a function of the proportion of the litter that was sired by a +/t male. The white line and the grey shaded area illustrate GLM predictions including the 95% CI. In all panels, the surface area of the dots represents the number of observations for a given *x, y* combination. Green color is used to depict mating crosses that involve +/+ females, red colors are used for mating crosses with *+/t* females.

Figure 2. Observed *t* haplotype frequency dynamics for the monandrous (green) and polyandrous (red) selection lines. Green lines show observed genotype frequency dynamics in the four monandrous selection lines. Red lines show frequency dynamics in the four polyandrous lines. Square dots and arrows depict mean and binomial standard errors of the observed frequencies in a given generation over all four selection lines of a given selection treatment, with the respective sample sizes given below/above. Thick lines and shaded areas show mixed-effect model predictions including 95% CI.